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Isolation and characterization of multilineage progenitor cells from the anterior cruciate ligament

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Introduction:

In this work, we specifically wished to characterize the differentiation potential of the cells that migrate out of the anterior cruciate ligament (ACL) compared to marrow derived mesenchymal stem cells (MSCs) in order to gain insights how to stimulate a more effective healing response of the ACL cells after injury.

Materials and Methods:

Cell isolation: ACL fibroblasts cells were isolated by explant cultures and MSCs were recovered by adhaerent culture of bone marrow aspirate which (informed consent and IRB approval). The recovered cells were placed in monolayer cultures in complete DMEM media. 2nd passage cells were used for all experiments. Cell surface antigens: FACS analyses have been performed using monoclonal antibodies for: CD14, CD29, CD31, CD34, CD44, CD45, CD90, CD105, CD106, CD133. Multilineage differentiation cultures: Osteogenesis was induced by monolayer cultures in complete DMEM supplemented with dexamethasone, s-glycerophosphate, ascorbate, and recombinant BMP-2 (25 ng/mL). The adipogenic phenotype was induced by supplementation with dexamethasone, insulin, indomethacine and IBMX in monolayer culture. Chondrogenesis was induced by aggregate cultures maintained in serum-free medium containing dexamethasone, ascorbate, proline, sodium pyruvate, and recombinant TGF-s1 (10 ng/mL). Negative control cultures were also maintained in the respective media without supplements, and all cultures were maintained for three weeks. Phenotype characterization: Lineage specific differentiation was analyzed by RT-PCR (COL I, II, IX, X, SOX-9, ALP, OC, Cbfa1, LPL, PPARg2) and histology and immunohistochemistry (H&E, Alcian Blue, ALP, Oil Red O, COL I, II, X).

Results:

FACS analyses revealed positive staining for the markers CD 29, 44, 90, 105, 106 for both cell types, but to a lesser extend in the ACL cells. All other markers were negative for both cell types. Following three weeks of differentiation culture, the ACL cells revealed a strong chondrogenic, adipogenic and osteogenic differentiation potential, such as the MSCs, as shown by the respective histological, immunohistochemical and RT-PCR analyses. In contrast, the respective

negative control cultures for the ACL cells and MSCs, which were maintained without any media supplements, were negative for the tissue specific markers.

Discussion:

The study was designed to characterize the cells migrating out of the bulk ACL tissue. Our study revealed an almost similar surface antigen expression profile compared to marrow derived-MSCs and an equivalent multilineage mesenchymal differentiation potential of these cells. This might be used in order to augment a favourable healing response toward healing of ACL defects by using e.g. growth factors. Global gene expression analyses (Affymetrix HG-U133_Plus) are underway to further characterize the stemness of the ACL cells on a molecular level.